SCANNING ATTACHMENT FOR A MICROSCOPE FOR CYTOSPECTROPHOTOMETRY

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The design of a scanning attachment to be fitted to a biological microscope for absorption spectrophotometry of biological objects is described.

Quantitative measurements in the study of biological micro-objects became feasible mainly through the introduction of cytophotometric analysis and, in particular, of absorption spectrophotometry, the object of which is to determine the concentrations of various substances in the cell and in its fragments. Cytophotometry is based on the Lambert–Beer Law, expressing the relationship between the optical density of an object and the concentration of absorbing material. However, this law is valid only for a homogeneous distribution of matter, and this is observed extremely rarely in biological objects. Application of the Lambert–Beer law to systems with a nonhomogeneous distribution of matter leads to considerable errors. To increase the accuracy of measurement, the object to be studied is broken up into areas with more or less uniform distribution, after which the mean or integral value of the optical density is determined. Systems giving automatic displacement of a ray of light over the test object are called scanning devices. The classification and constructional details of scanning instruments are given in Katys's monograph [2], and as applied to the analysis of microscopic objects, they have been described by other workers [1, 3, 4].

When developing a scanning instrument for the microscope of a cytospectrophotometer, the choice fell on an optical-mechanical system with a refracting scanning element. Such a system, with high linearity of scanning, enables the scanning instrument to be designed as an attachment to the biological microscope, to which it is fitted instead of the objective holder. An oscillating transparent plane-parallel plate is used as the element deflecting the beam. As the beam passes through the plate, which lies at a certain angle to it, the beam is displaced by an amount h, determined by the equation

$$h = \frac{\delta}{\cos\beta} \cdot \sin(\alpha - \beta),$$

where α is the angle of incidence of the beam, β the angle of refraction, and δ the thickness of the plate.

The following conclusions can be drawn from analysis of this equation:

1. If the plate oscillates within the limit of ±10°, displacement of the beam is, for practical purposes, proportional to the angle of incidence of the beam.

2. Scanning can also be carried out if the angle of inclination of the plate remains unchanged, for which purpose the refractive index or the thickness of the plate must be changed (continuously or discretely).

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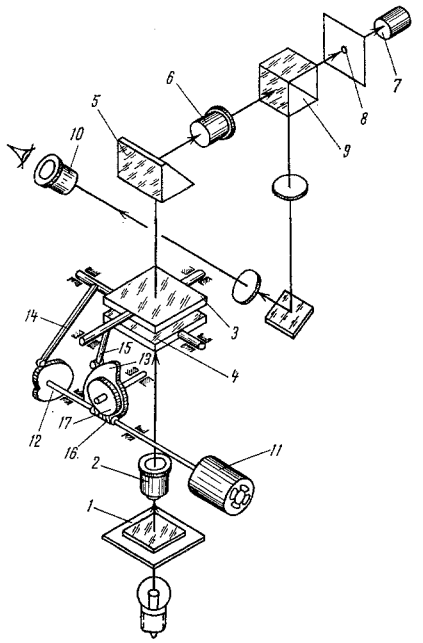

Fig. 1. Theoretical design of microscope with scanning attachment for cytospectrophotometry (explanation in text).

3. During work in the visible part of the spectrum, because of differences in the refractive index of beams of different wavelengths, the beam will be broken up, and this must reduce, to some extent, the resolving power of the system. This defect is not present if the investigation is carried out in monochromatic light.

In the first type of attachment to the MBI-6 biological microscope, scanning along the line and frame is achieved by means of a single plane-parallel refracting plate, which oscillates in two mutually perpendicular planes. The frequency of oscillation was chosen so that during movement along the frame the beam described 30 lines. The trajectory of movement of the beam was of the sawtooth variety, so that loss of time during the return course of the beam was eliminated. The size of the scanned frame can be changed by fitting a plate of different thickness from an available series.

In the most recent designs, the kinematic drive system has been simplified considerably by dividing the function between two plane-parallel plates, placed behind the objective of the microscope. One plate made frequent oscillations and displaced the beam along the line, while the other plate, revolving around its axis perpendicular to the plane of oscillation of the first plate, moved the beam along the frame. Both plates were replaceable, so that not only the size of the scanning frame, but also the ratio between its sides could be changed. The scanning trajectory, as in the first type, was of the sawtooth variety.

The theoretical scheme of the MBI-6 microscope for cytospectrophotometry, with the scanning attachment fitted, is shown in Fig. 1.

From the specimen, illuminated by transmitted light and placed on the stage of the microscope (1), the beams pass through the object (2) and plane-parallel plates (3) and (4) and fall on the prism (5), which directs the beams into the focal plane of the camera oculars (6) where an image is obtained of the objective. The camera oculars (6) project the image onto a type FÉU photoelectronic multiplier (7) through an apperture (8) in an opaque screen. A prism, consisting of a cube (9) with color-selective coating is placed between the camera oculars and the FÉU instrument, directing a small part of light into the optical system of the observation tube (10).

The drive for the oscillatory movement of the plane-parallel plates (3) and (4) consists of a synchronized electric motor (11) with inbuilt reducing gear and cams (12) and (13), which interact with spring-loaded levers (14) and (15). The cam (12), fitted to the worm shaft (16) of the reducing gear, is cardioid in shape, thus giving a uniform angular displacement to the plate (3) and, consequently, a constant scanning speed of the image along the line. The angle of inclination of the plate (3) with the horizontal is 8° in both directions. During scanning of the frame the plate (3) makes 30 complete oscillations, so that 60 lines can be obtained in the frame. The profile of the cam (13) fixed to the shaft of the worm-driven pinion (17) is so designed as to give linear angular displacement of the plate (4) during scanning of the line and its rapid return to the original position in a time of about 3% of its period.

Hence, during oscillation of the two plates the FÉU successively examines the frame through the apperture (8). The size of the scanning frame can be varied at will within wide limits, sufficient to allow the study of any cytological object, simply by changing the thickness of the plate and the magnification of the microscope. The duration of scanning, which is controlled mainly by the possibility of visual control over the process, is 18 sec.

A synchronous FD-54 electric motor is used in the attachment, and its inbuilt reducing gear gives a velocity of 102 rpm at the output. The parameters of the worm-reducing gear are as follows: modulus 0.3, transmission ratio $^1/_{34}$. The attachment is supplied with a set of transparent plane-parallel plates made of K8 optical glass, 16 x 16 mm in area and from 1 to 7 mm, at 1 mm intervals, in thickness. Of all the photoelectronic multipliers tested, the best results were obtained with the FÉU-27.

The scanning attachment as designed above has been used in a recording cytospectrophotometer built at the Institute of Chemical Physics, Academy of Sciences of the USSR. Tests with this device have demonstrated the high reliability of the instrument and the stability of the linearity of its scanning over a long period of time. The simplicity of design of the attachment, and the fact that it can be used over a wide region of the spectrum enable it to be recommended for wide use in cytological investigation.

The Institute of Chemical Physics, Academy of Sciences of the USSR, will send drawings of these scanning attachments to interested organizations.

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